PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 4239-66176	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US03/20367	International filing date *(day/month/year)* 26 June 2003 (26.06.2003)	Priority date *(day/month/year)* 28 June 2002 (28.06.2002)

International Patent Classification (IPC) or national classification and IPC

IPC(7): C07H 21/04; C07K 16/00; A61K 39/395; G01N 33/53 and US Cl.: 536/23.53; 435/320.1, 7.1; 530/387.1, 388.85, 391.1; 424/133.1, 156.1, 181.1

Applicant

THE GOVERNMENT OF THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY OF THE DEPARTMENT OF HEALTH AND HUMAN SERVICES

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of report with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05 December 2003 (05.12.2003)	21 October 2004 (21.10.2004)
Name and mailing address of the IPEA/US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Jackie Bell-Harris for Larry R. Helms Telephone No. (571) 272-1600

Form PCT/IPEA/409 (cover sheet)(July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US03/20367

I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed.

☒ the description:
pages 1-55 as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the claims:
pages NONE, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages 56-63, filed with the letter of 21 July 2004 (21.07.2004).

☒ the drawings:
pages 1-9, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the sequence listing part of the description:
pages 1-5, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in printed form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages NONE

☐ the claims, Nos. NONE

☐ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US03/20367

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-67	YES
	Claims NONE	NO
Inventive Step (IS)	Claims 1-67	YES
	Claims NONE	NO
Industrial Applicability (IA)	Claims 1-67	YES
	Claims NONE	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-67 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a variant humanized CC49 antibody with a non-conservative substitution in L-CDR3 with high binding affinity compared to a parent CC49 antibody.

---------- NEW CITATIONS ----------

We claim:

1. A variant humanized CC49 antibody, comprising:

a light chain complementarity determining region (L-CDR)1, a L-CDR2, and a L-CDR3, a heavy chain complementarity determining region (H-CDR)1, a H-CDR2, and a H-CDR3,

wherein a L-CDR3 of the variant humanized CC49 antibody or of a functional fragment of the variant humanized CC49 antibody comprises a non-conservative amino acid substitution, and wherein the variant humanized CC49 antibody has a high binding affinity for TAG-72, compared to a parent CC49 antibody.

2. The variant antibody of claim 1, wherein the non-conservative substitution is a tyrosine to proline substitution.

3. The variant antibody of claim 1, wherein the non-conservative substitution is at position 91.

4. The variant antibody of claim 1, wherein the non-conservative substitution is at a position that corresponds to a ligand contact residue.

5. The variant antibody of claim 1, wherein the functional fragment is an Fab fragment, an Fv fragment, or an $F(ab')_2$ fragment.

6. The variant antibody of claim 1, wherein the L-CDR1 and L-CDR2 are a human antibody L-CDR1 and L-CDR2, respectively.

7. The variant antibody of claim 1, wherein the L-CDR3, H-CDR1, H-CDR2, and H-CDR3 are from a murine CC49 antibody.

8. The variant antibody of claim 1, wherein the high binding affinity is at least about 1.2×10^{-8} M.

9. The variant antibody of claim 8, wherein the high binding affinity is at least about 1.5×10^{-8}, about 2.0×10^{-8}, about 2.5×10^{-8}, about 3.0×10^{-8}, about 3.5×10^{-8}, about 4.0×10^{-8}, about 4.5×10^{-8}, or about 5.0×10^{-8} M.

10. The variant antibody of claim 1, wherein the antibody is minimally immunogenic.

11. The variant antibody of claim 1, wherein the antibody further comprises an effector molecule.

12. The variant antibody of claim 11, wherein the effector molecule is a detectable label.

13. The variant antibody of claim 12, wherein the detectable label comprises a radioactive isotope, an enzyme substrate, a co-factor, a ligand, a chemiluminescent agent, a fluorescent agent, a hapten, or an enzyme.

14. The variant antibody of claim 11, wherein the effector molecule is a toxin.

15. The variant antibody of claim 14, wherein the toxin is a chemotherapeutic drug, a radioactive isotope, a bacterial toxin, a viral toxin, a cytokine or a venom protein.

16. The variant antibody of claim 1, further comprising at least one additional non-conservative amino acid substitution in the L-CDR1.

17. The variant antibody of claim 1, further comprising at least one additional non-conservative amino acid substitution in the L-CDR2, or L-CDR3.

18. The variant antibody of claim 1, further comprising at least one non-conservative amino acid substitution in the H-CDR1.

19. The antibody of claim 1, further comprising at least one non-conservative amino acid substitution in the H-CDR2, or H-CDR3.

20. A humanized CC49 antibody, wherein a nucleic acid sequence encoding the antibody has an ATCC Accession number comprising ATCC Accession number PTA-4182 or ATCC Accession number PTA-4183.

21. A nucleic acid molecule encoding the variant humanized monoclonal antibody of claim 1.

22. A vector comprising the nucleic acid of claim 21.

23. A variant humanized CC49 antibody, comprising:

a variable light framework region and a variable heavy framework region of a human antibody;

a light chain complementarity determining region (L-CDR)1, a L-CDR2, a L-CDR3, a heavy chain complementarity determining region (H-CDR)1, a H-CDR2, and a H-CDR3, wherein at least one complementarity determining region (CDR) is a human antibody CDR and remaining CDRs are murine CC49 antibody CDRs;

a non-conservative substitution of a first residue, wherein the first residue is in the L-CDR3 of the variant antibody; and

a substitution of a second residue, wherein the second residue is in a any L-CDR or H-CDR of the variant antibody;

wherein the humanized CC49 antibody has a high binding affinity for TAG-72 and is minimally immunogenic, compared to a parent CC49 antibody.

24. The variant antibody of claim 23, wherein the non-conservative substitution of the first residue is a tyrosine to proline substitution.

25. The variant antibody of claim 23, wherein the non-conservative substitution of the first residue is at position 91.

26. The variant antibody of claim 25, wherein the non-conservative substitution of the first residue at position 91 is a tyrosine to proline substitution.

27. The variant antibody of claim 23, wherein the antibody further comprises an effector molecule.

28. The variant antibody of claim 27, wherein the effector molecule is a detectable label.

29. The variant antibody of claim 28, wherein the detectable label comprises a radioactive isotope, an enzyme substrate, a co-factor, a ligand, a chemiluminescent agent, a fluorescent agent, a hapten, or an enzyme.

30. The variant antibody of claim 27, wherein the effector molecule is a toxin.

31. The variant antibody of claim 30, wherein the toxin is a chemotherapeutic drug, a radioactive isotope, a bacterial toxin, a viral toxin, a cytokine or a venom protein.

32. A method of detecting a TAG-72-expressing tumor in a subject, comprising:

contacting a sample obtained from the subject with the variant antibody of claim 1 for a sufficient amount of time to form an immune complex; and

detecting the presence of the immune complex, wherein the presence of the immune complex demonstrates the presence of the TAG-72-expressing tumor.

33. The method of claim 32, wherein the tumor is a colorectal tumor, a gastric tumor, a pancreatic tumor, a breast tumor, a lung tumor, an adenocarcinoma, or an ovarian tumor.

34. The method of claim 32, wherein the variant antibody further comprises an effector molecule.

35. The method of claim 34, wherein the effector molecule is a detectable label.

36. The method of claim 35, wherein the detectable label comprises a radioactive isotope, an enzyme substrate, a co-factor, a ligand, a chemiluminescent agent, a fluorescent agent, a hapten, or an enzyme.

37. The method of claim 32, further comprising contacting the variant antibody with a secondary antibody.

38. The method of claim 37, wherein the secondary antibody further comprises a detectable label.

39. A method of detecting a TAG-72-expressing tumor in a subject, comprising:

administering the variant antibody of claim 1 to the subject for a sufficient amount of time to form an immune complex; and

detecting the presence of the immune complex, wherein the presence of the immune complex demonstrates the presence of the TAG-72-expressing tumor.

40. The method of claim 39, wherein the variant antibody further comprises an effector molecule.

41. The method of claim 40, wherein the effector molecule is a detectable label.

42. The method of claim 41, wherein the detectable label comprises a radioactive isotope, an enzyme substrate, a co-factor, a ligand, a chemiluminescent agent, a fluorescent agent, a hapten, or an enzyme.

43. The method of claim 39, wherein the tumor is a colorectal tumor, a gastric tumor, a pancreatic tumor, a breast tumor, a lung tumor, an adenocarcinoma, or an ovarian tumor.

44. A method of treating a subject having a tumor that expresses TAG-72, comprising administering to the subject a therapeutically effective amount of the variant antibody of claim 1, wherein administering the therapeutically effective amount of the variant antibody of claim 1 inhibits the growth of the tumor or reduces the size of the tumor, thereby treating the subject.

45. The method of claim 44, wherein the administration of a therapeutically effective amount of the variant antibody of claim 1 does not elicit a human anti-murine antibody response in a subject.

46. The method of claim 44, wherein the tumor is a colorectal tumor, a gastric tumor, a pancreatic tumor, a breast tumor, a lung tumor, an adenocarcinoma, or an ovarian tumor.

47. The method of claim 44, wherein the variant antibody further comprises an effector molecule.

48. The method of claim 47, wherein the effector molecule is a toxin.

49. The method of claim 48, wherein the toxin is a chemotherapeutic drug, a radioactive isotope, a bacterial toxin, a viral toxin, a cytokine, or a venom protein.

50. The method of claim 49, wherein the variant antibody comprising a radioactive isotope is used in radioimmunotherapy.

51. A method of treating a subject having a tumor that expresses TAG-72, comprising:

administering the variant antibody of claim 1 to the subject for a sufficient amount of time to form an immune complex, wherein the variant antibody comprises a radioactive isotope;

detecting the presence of the immune complex with a hand-held gamma counter, wherein the presence of the immune complex demonstrates the presence of the TAG-72-expressing tumor; and

removing the tumor surgically, thereby treating the subject.

52. A pharmaceutical composition comprising a therapeutically effective amount of the variant antibody of claim 1 in a pharmaceutically acceptable carrier.

53. A kit, comprising a container comprising the variant antibody of claim 1.

54. The kit of claim 53, further comprising a container containing an antigen, a container containing a secondary antibody conjugated to a chemical compound, instructions for using the kit, or any combination thereof.

55. The variant antibody of claim 1, wherein the L-CDR1, L-CDR2, L-CDR3, H-CDR1, H-CDR2, and H-CDR3 are the parent CC49 antibody L-CDR1, L-CDR2, L-CDR3, H-CDR1, H-CDR2, and H-CDR3, respectively.

56. The variant antibody of claim 1, wherein the parent humanized CC49 antibody is HuCC49V10.

57. The variant antibody of claim 1, wherein the non-conservative substitution is a tyrosine to proline substitution at position 91.

58. The variant antibody of claim 57, further comprising a substitution of a second residue, wherein the second residue is in the L-CDR1, L-CDR2, or L-CDR3.

59. The variant antibody of claim 58, wherein the substitution of the second residue is in L-CDR1.

60. The variant antibody of claim 59, wherein the substitution of the second residue is at position 27b of the L-CDR1.

61. The variant antibody of claim 60, wherein the substitution of the second residue is a valine to leucine substitution.

62. The variant antibody of claim 23, wherein the parent CC49 antibody is HuCC49V10.

63. The variant antibody of claim 23, wherein the substitution of the second residue is in the L-CDR1, L-CDR2, or L-CDR3 of the variant antibody.

64. The variant antibody of claim 63, wherein the substitution of the second residue is in L-CDR1.

65. The variant antibody of claim 64, wherein the substitution of the second residue is at position 27b of the L-CDR1.

66. The variant antibody of claim 65, wherein the substitution of the second residue is a valine to leucine substitution.

67. The variant antibody of claim 23, wherein the non-conservative substitution of the first residue at position 91 is a tyrosine to proline substitution, the substitution of the second residue at position 27b is a valine to leucine substitution, the L-CDR1, L-CDR2, L-CDR3, H-CDR1, H-CDR2, and H-CDR3 are the parent CC49 antibody L-CDR1, L-CDR2, L-CDR3, H-CDR1, H-CDR2, and H-CDR3, respectively, and the parent CC49 antibody is HuCC49V10.